

WHAT IS CLAIMED IS:

1. A method for creating a uniform vascular wound in a zebrafish larva or zebrafish, comprising:

5 (a) subjecting a zebrafish larva to laser irradiation in an amount and for a period of time effective to cause a uniform vascular wound in said zebrafish larva; or

10 (b) exposing a zebrafish to water containing sodium hydroxide in an amount and for a period of time effective to cause a uniform vascular wound detectable in the gills of said zebrafish.

15 2. The method of claim 1, comprising subjecting a zebrafish larva to laser irradiation in an amount and for a period of time effective to cause a uniform vascular wound in said zebrafish larva.

20 3. The method of claim 2, wherein said zebrafish larva is a zebrafish larva three to five days postfertilization.

4. The method of claim 2, wherein said zebrafish larva is anesthetized.

25 5. The method of claim 2, wherein said zebrafish larva is immobilized in agarose.

30 6. The method of claim 2, wherein said laser irradiation is applied to a major blood vessel of said zebrafish larva to cause a uniform injury in said blood vessel.

7. The method of claim 6, wherein said laser irradiation is applied to a major artery of said zebrafish larva.

8. The method of claim 6, wherein said laser irradiation is applied to a major vein of said zebrafish larva.

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9. The method of claim 6, further comprising measuring the time to occlusion in the injured blood vessel.

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10. The method of claim 1, comprising exposing a zebrafish to water containing sodium hydroxide in an amount and for a period of time effective to cause a uniform vascular wound detectable in the gills of said zebrafish.

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11. The method of claim 10, wherein said zebrafish is an adult zebrafish.

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12. The method of claim 10, further comprising measuring the time to bleeding in the gills of said zebrafish.

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13. The method of claim 1, further comprising contacting said zebrafish larva or zebrafish with a candidate substance and testing the ability of said candidate substance to alter the vascular wound created in said zebrafish larva or zebrafish.

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14. The method of claim 1, wherein said zebrafish larva or zebrafish is a mutant or genetically engineered zebrafish larva or zebrafish.

15. The method of claim 14, wherein zebrafish larva or zebrafish is one of a population of mutant zebrafish larvae or zebrafish produced by large-scale mutagenesis.

16. A method for creating a uniform vascular injury in a zebrafish larva, comprising
subjecting a zebrafish larva to laser irradiation in an amount and for a period of time effective
to cause a reproducible thrombus in a major artery or a major vein of said zebrafish larva,
wherein said reproducible thrombus is reversible so that circulation returns at the site of
injury.
17. A method for creating a uniform vascular injury in a zebrafish, comprising exposing
an adult zebrafish to water containing sodium hydroxide in an amount and for a period of
time effective to cause a reproducible visible hemorrhage in the gills of said zebrafish.
18. A method for measuring coagulation activity in a zebrafish blood sample, comprising
collecting a zebrafish blood sample in a heparinized capillary tube and determining the time
required for significant lysis of red cells in said blood sample.
19. The method of claim 18, comprising collecting a zebrafish blood sample in a
heparinized capillary tube, centrifuging said capillary tube to separate red cells from plasma,
and determining the time required for a significant red color to develop in said plasma.
20. The method of claim 18, wherein said blood sample is collected from a zebrafish
following exposure to a candidate substance and wherein the ability of said candidate
substance to alter the coagulation activity of said blood sample is determined.
21. The method of claim 18, wherein said blood sample is collected from a mutant or
genetically engineered zebrafish.
22. A method for measuring the clotting activity of a zebrafish blood sample, comprising
collecting a zebrafish blood sample in a heparinized capillary tube, centrifuging said capillary

tube to separate red cells from plasma, and determining the time required for significant red cell lysis by measuring the time for a significant red color to develop in said plasma following lysis of the red cells.

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23. A method for analyzing coagulation in zebrafish, comprising:

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(a) subjecting a zebrafish larva to an amount of laser irradiation effective to cause a uniform vascular wound and measuring the time to coagulation in said wound;

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(b) exposing a zebrafish to water containing an amount of sodium hydroxide effective to cause a uniform vascular wound in the gills of said zebrafish and measuring the time to coagulation in said wound; or

(c) collecting a zebrafish blood sample in a heparinized capillary tube and measuring the time required for significant red cell lysis in said sample.

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24. The method of claim 23, comprising subjecting a zebrafish larva to an amount of laser irradiation effective to cause a uniform vascular wound and measuring the time to coagulation in said wound.

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25. The method of claim 23, comprising exposing a zebrafish to water containing an amount of sodium hydroxide effective to cause a uniform vascular wound in the gills of said zebrafish and measuring the time to coagulation in said wound.

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26. The method of claim 23, comprising collecting a zebrafish blood sample in a heparinized capillary tube and measuring the time required for significant red cell lysis in said sample.

27. The method of claim 23, wherein coagulation is analyzed in a zebrafish larva or zebrafish exposed to a candidate substance, or in a blood sample therefrom, and the ability of said candidate substance to alter coagulation is determined.

5 28. The method of claim 27, wherein coagulation is analyzed in a zebrafish larva exposed to a candidate substance and the ability of said candidate substance to alter the time to occlusion in an injured blood vessel of said zebrafish larva is determined.

10 29. The method of claim 27, wherein coagulation is analyzed in a zebrafish exposed to a candidate substance and the ability of said candidate substance to alter the bleeding time in the gills of said zebrafish is determined.

15 30. The method of claim 27, wherein coagulation is analyzed in a blood sample collected from a zebrafish exposed to a candidate substance and the ability of said candidate substance to alter red cell lysis in said blood sample is determined.

20 31. The method of claim 23, wherein coagulation is analyzed in a mutant or genetically engineered zebrafish larva or zebrafish, or in a blood sample therefrom.

25 32. The method of claim 31, wherein coagulation is analyzed in one of a population of mutant zebrafish larvae or zebrafish produced by large-scale mutagenesis, or in a blood sample therefrom.

30 33. The method of claim 31, wherein coagulation is analyzed in a zebrafish larva or zebrafish comprising a mutation in a selected gene, or in a blood sample therefrom.

34. The method of claim 33, wherein coagulation is analyzed in a genetically engineered zebrafish larva or zebrafish comprising an antisense oligonucleotide, or derivative thereof, which specifically inhibits a selected zebrafish gene, or in a blood sample therefrom.

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35. The method of claim 31, wherein coagulation is analyzed in a zebrafish larva or zebrafish that expresses a mutant gene or an exogenous transgene, or in a blood sample therefrom, and the effect of the mutant gene or exogenous transgene on coagulation is determined.

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36. A method for identifying a candidate substance that alters thrombosis, comprising contacting zebrafish larvae or zebrafish with a candidate substance and determining the ability of said candidate substance to change the coagulation time in zebrafish blood, wherein
15 an ability to change the coagulation time in zebrafish blood is measured by:

- (a) creating laser irradiation vascular wounds in zebrafish larvae and measuring the occlusion time in said wounds in the presence and absence of said candidate substance;
- 20 (b) creating sodium hydroxide-induced vascular gill wounds in zebrafish and measuring the coagulation time in said wounds in the presence and absence of said candidate substance; or
- 25 (c) collecting zebrafish blood samples in heparinized capillary tubes and measuring the time required for significant red cell lysis in samples from zebrafish in the presence and absence of said candidate substance;

wherein a candidate substance that changes said coagulation time is indicative of a candidate
30 substance that alters thrombosis.

37. The method of claim 36, wherein said ability to change the coagulation time in zebrafish blood is measured by creating laser irradiation vascular wounds in zebrafish larvae

and measuring the occlusion time in said wounds in the presence and absence of said candidate substance.

5 38. The method of claim 36, wherein said ability to change the coagulation time in zebrafish blood is measured by creating sodium hydroxide-induced vascular gill wounds in zebrafish and measuring the coagulation time in said wounds in the presence and absence of said candidate substance.

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39. The method of claim 36, wherein said ability to change the coagulation time in zebrafish blood is measured by collecting zebrafish blood samples in heparinized capillary tubes and measuring the time required for significant red cell lysis in samples from zebrafish in the presence and absence of said candidate substance.

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40. The method of claim 36, wherein an ability to increase said coagulation time is indicative of a candidate anticoagulant.

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41. The method of claim 36, wherein an ability to decrease said coagulation time is indicative of a candidate coagulant.

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42. The method of claim 36, further comprising purifying a candidate substance so identified.

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43. A method for identifying a candidate substance that alters thrombosis, comprising creating a uniform vascular wound in a zebrafish larva using laser irradiation and testing a candidate substance for the ability to alter the occlusion time in said wound in comparison to the occlusion time in a wound in a zebrafish larva in the absence of said candidate substance.

44. A method for identifying a candidate substance that alters thrombosis, comprising creating a uniform vascular wound detectable in the gills of a zebrafish by exposure to sodium hydroxide and testing a candidate substance for the ability to alter the coagulation time in said wound in comparison to the coagulation time in a wound in a zebrafish in the absence of said candidate substance.

45. A method for identifying a candidate substance that alters thrombosis, comprising collecting in a heparinized capillary tube a blood sample from a zebrafish exposed to a candidate substance and determining the red cell lysis time in said blood sample in comparison to the red cell lysis time in a counterpart blood sample collected from a zebrafish in the absence of said candidate substance.

46. A method for identifying a gene associated with coagulation, comprising creating a mutant zebrafish larvae or zebrafish comprising a mutation in a gene and determining the effect of the mutation on coagulation time in zebrafish blood, wherein the effect of the mutation on coagulation time in zebrafish blood is measured by:

(a) creating laser irradiation vascular wounds in zebrafish larvae and measuring the occlusion time in said wounds in the presence and absence of said mutation;

(b) creating sodium hydroxide-induced vascular gill wounds in zebrafish and measuring the coagulation time in said wounds in the presence and absence of said mutation; or

(c) collecting zebrafish blood samples in heparinized capillary tubes and measuring the time required for significant red cell lysis in samples from zebrafish in the presence and absence of said mutation;

wherein identifying a mutation that changes said coagulation time is indicative of a gene associated with coagulation.

47. The method of claim 46, wherein said mutant zebrafish larvae or zebrafish is one of a population of mutant zebrafish larvae or zebrafish produced by large-scale mutagenesis.

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48. The method of claim 46, further comprising mapping a gene so identified.

49. The method of claim 46, further comprising isolating a gene so identified.

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50. A method for identifying a gene associated with coagulation, comprising mutagenizing a zebrafish population to generate a plurality of mutant zebrafish larvae or zebrafish and selecting a mutant with an altered coagulation time, thereby identifying a gene associated with coagulation; wherein a mutant with an altered coagulation time is selected
15 by:

(a) creating laser irradiation vascular wounds in a plurality of zebrafish larvae, measuring the occlusion time in said wounds and identifying a mutant with an
20 altered occlusion time;

(b) creating sodium hydroxide-induced vascular gill wounds in a plurality of zebrafish, measuring the coagulation time in said wounds and identifying a mutant with an altered coagulation time; or

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(c) collecting a plurality of zebrafish blood samples in heparinized capillary tubes, measuring the red cell lysis time in said samples and identifying a mutant with an altered red cell lysis time.

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51. The method of claim 50, wherein a mutant with an altered coagulation time is selected by creating laser irradiation vascular wounds in a plurality of zebrafish larvae, measuring the occlusion time in said wounds and identifying a mutant with an altered occlusion time.

52. The method of claim 50, wherein a mutant with an altered coagulation time is selected by creating sodium hydroxide-induced vascular gill wounds in a plurality of zebrafish, measuring the coagulation time in said wounds and identifying a mutant with an altered coagulation time.

53. The method of claim 50, wherein a mutant with an altered coagulation time is selected by collecting a plurality of zebrafish blood samples in heparinized capillary tubes, measuring the red cell lysis time in said samples and identifying a mutant with an altered red cell lysis time.

54. The method of claim 50, wherein said plurality of mutant zebrafish larvae or zebrafish are generated by large-scale chemical mutagenesis.

55. The method of claim 50, further comprising mapping a gene so identified.

56. The method of claim 50, further comprising isolating a gene so identified.

57. The method of claim 50, further comprising sequencing a gene so identified.

58. The method of claim 50, further comprising identifying the human homologue of a gene so identified.